TECHNICAL DATA SHEET

RHAPSODY AGAR®

ENUMERATION OF *PSEUDOMONAS* SPP.

1 INTENDED USE

RHAPSODY Agar® is a selective medium used for the enumeration of *Pseudomonas* spp. in food products and environmental samples.

This method is certified NF VALIDATION according to the validation protocol ISO 16140-2:2016, for the enumeration of *Pseudomonas spp.* in meat products.

BKR 23/09-05/15 A,
METHODES ALTERNATIVES D'ANALYSE
POUR L'AGROALIMENTAIRE
Certifié par AFNOR Certification http://nf-validation.afnor.org

Refer to the certificate available on the NF VALIDATION website for the end of validity date of the method.
The reference method used for the validation is the standard NF EN ISO 13720 : 2010.

This method is also certified NF VALIDATION according to the validation protocol ISO 16140-2:2016, for the enumeration of *Pseudomonas spp.* in dairy products.

BKR 23/09-05/15 B,
METHODES ALTERNATIVES D'ANALYSE
POUR L'AGROALIMENTAIRE
Certifié par AFNOR Certification http://nf-validation.afnor.org

Please refer to the certificate available on the NF VALIDATION website for the validity end date of the method.
The reference method used for the validation is the XP ISO/TS 11059:2009 standard.

The validated method allows the enumeration in 48 ± 3 hours without confirmation for meat products and dairy products.

2 PRINCIPLES

The peptones constitute nutritive substrates necessary for the rapid growth of *Pseudomonas*.

The chromogenic substrate contained in the medium is hydrolyzed by all *Pseudomonas*. As a result, colonies present a light blue to blue-green coloration.

The selective system and the cephalosporin insure the inhibition of most of the secondary flora.

3 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1010 mL of complete media:

- Polypeptone ..... 28,9 g
- Buffered system ..... 7,0 g
- Sodium chloride ..... 5,0 g
- Selective system ..... 5,5 g
- Chromogenic mixture ..... 0,2 g

- Bacteriological agar ..... 15,0 g

pH of the ready-to-use media at 25°C: 7,0 ± 0,2.

4 PREPARATION

Preparation from dehydrated media:

- Dissolve 61,6 g of dehydrated media (BK214) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense in vials of 100 mL or 200mL.
- Sterilize in an autoclave at 110 °C for 15 minutes.
- Cool and maintain the media in a molten state at 44-47 °C.

✓ **Reconstitution : 61,6 g/L**

✓ **Sterilization : 15 min at 110 °C**

Rehydration of freeze-dried supplement:

- Rehydrate the selective supplement (BS089) by adding aseptically 5 mL of sterile distilled water.
- Turn end-over-end to dissolve. Avoid frothing the solution

Preparation from complete media:

- Melt the base medium (if it's prepared in advance) until it is completely reliquefied.
- Cool and maintain at 44-47°C.
- Aseptically add 1 mL of reconstituted RHAPSODY Agar® Supplement (BS08908) to each 100 mL vial of base medium.
- Homogenize thoroughly.
- Pour the appropriate amount of complete media into sterile Petri dishes and let solidify on a cool surface.

5 INSTRUCTIONS FOR USE

Respect the good laboratory practices.
Refer to the NF EN ISO 7218 standard for plating, colony counting and for calculations and expression of results.
Prepare the mother suspension of the sample and the decimal dilutions according to the rules defined in the corresponding ISO 6887 standards

- To the surface of plates prepared as above or pre-poured plates (BM16708), transfer 0.1 mL of the sample to be tested and its serial dilution.
- Spread the inoculum on the surface with a sterile triangle or « hockey stick ».
- Incubate at 30 ± 1 °C for 48 hours ± 3 hours.

✓ **Inoculation : 0,1 mL on surface**

✓ **Incubation : 48 ± 3 h at 30 ± 1 °C**

NOTES :

- The method is also validated for the Spiral inoculation technique. The plating can be in logarithmic mode of 50 or 100µL.
- The enumeration limit can be reduced by a factor of 10 by inoculating 1 mL of initial suspension on to the surface of 3 Petri dishes (Ø 90 mm) or 1 single Petri dish (Ø 140 mm).

For organizational reasons in the laboratories, the incubation is validated between 45 and 72 hours.

6 RESULTS

Characteristic colonies show light blue to blue-green coloration.
Colony diameter and color intensity may vary according to *Pseudomonas* species.

See ANNEX 1: PHOTO SUPPORT.

7 QUALITY CONTROL

Dehydrated media: beige powder, free-flowing and homogeneous.
Complete media: amber agar.

Typical culture response on complete media after 48 hours of incubation at 30 °C:

Microorganisms		Growth (Productivity Ratio : P_R)
Pseudomonas aeruginosa	WDCM 00025	$P_R \geq 50\%$
Pseudomonas putida	WDCM 00117	$P_R \geq 50\%$
Escherichia coli	WDCM 00013	Inhibited, score 0

8 STORAGE / SHELF LIFE

Dehydrated media: 2-30 °C.
Freeze-dried supplements: 2-8 °C.
Pre-poured media: 2-8 °C.
The expiration dates are indicated on the labels.

Prepared base media in vial (*): 30 days at 2-8 °C.
Prepared media in plates (*): 30 days at 2-8 °C.
(*): Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

9 PACKAGING

Pre-poured media in Petri plates (∅ 90 mm) :
20 plates BM16708

Dehydrated media
500 g bottle BK214HA

RHAPSODY Selective Supplement
10 vials x qsp 500 mL BS08908

10 BIBLIOGRAPHY

XP ISO/TS 11059. October 2009. Milk and milk products - Method for the enumeration of *Pseudomonas* spp.

NF EN ISO 13720. November 2010. Meat and meat products - Enumeration of presumptive *Pseudomonas* spp.

NF EN ISO 7218. October 2007. Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations.

NF EN ISO 16140-2. September 2016. Microbiology of the food chain - Method validation - Part 2: protocol for the validation of alternative (proprietary) methods against a reference method.

NF EN ISO 6887-1. June 2017. Microbiology of the food chain - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 1: General rules for the preparation of the initial suspension and decimal dilutions.

NF EN ISO 6887-2. June 2017. Microbiology of the food chain - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 2: Specific rules for the preparation of meat and meat products.

NF EN ISO 6887-5. June 2017. Microbiology of the food chain - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 5 : specific rules for the preparation of milk and milk products.

11 ADDITIONAL INFORMATION

RHAPSODY Agar® is a registered trademark of BIOKAR DIAGNOSTICS (division of SOLABIA S.A.S).

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning

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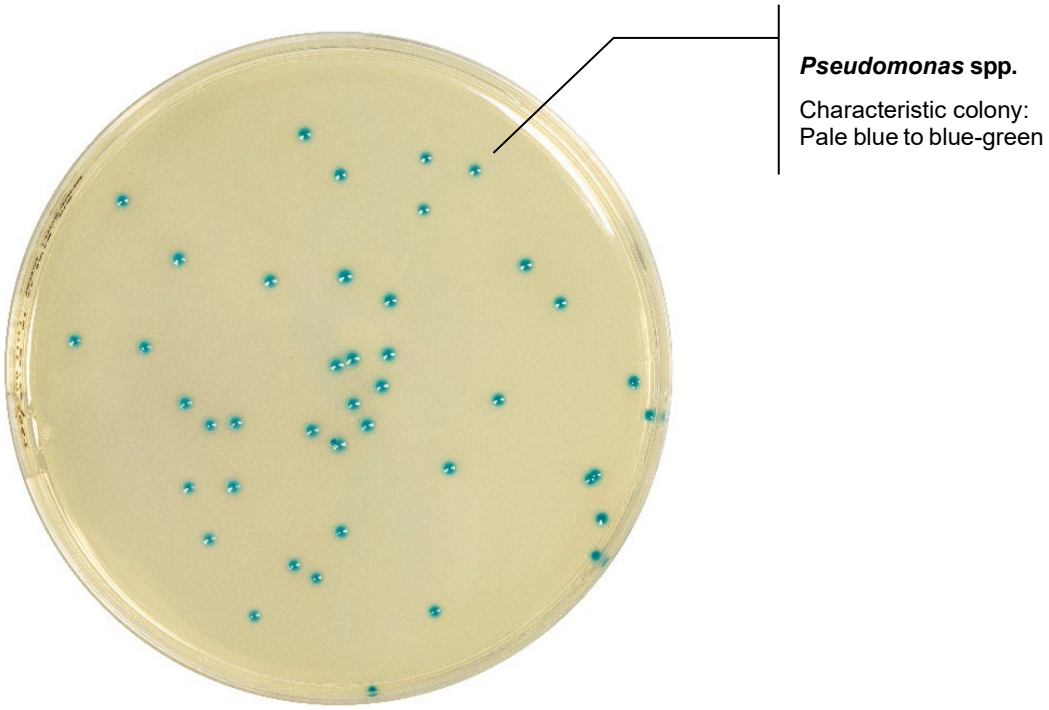
RHAPSODY Agar®

Detection and enumeration of *Pseudomonas* spp.

Results:

Growth obtained after 48 hours of incubation at 30 °C.



The size of the colonies and the intensity of the blue to blue-green coloration can vary depending on the species of *Pseudomonas* found on the plates.